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=> s Alkaline lipase and Vibrio metschnikovii
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L1 7 ALKALINE LIPASE AND VIBRIO METSCHNIKOVII

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L2 ANSWER 1 OF 5 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 1
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TITLE: **ALKALINE LIPASE FROM
VIBRIO METSCHNIKOVII RH530 N-4-8
AND NUCLEOTIDE SEQUENCE ENCODING THE SAME**
INVENTOR(S): Jhon; Sung Hoo, Seoul, KR
Jin; Ghee Hong, Seoul, KR
Lee; Hyun Hwan, Yongin-City, KR
Rho; Hyune Mo, Seoul, KR
PATENT ASSIGNEE(S): Unassigned
AGENT: Cantor Colburn LLP, 55 Griffin South Road,
Bloomfield, CT, 06002, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2004009570	A1	20040115
APPLICATION INFORMATION:	US 2003-603260		20030624

	NUMBER	DATE
PRIORITY APPLN. INFO.:	KR 2002-35410	20020624
FAMILY INFORMATION:	US 2004009570	20040115
DOCUMENT TYPE:	Utility	

FILE SEGMENT: Patent Application - First Publication
CHEMICAL
APPLICATION

NUMBER OF CLAIMS: 12 10 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 shows a recombinant vector pHL1 containing 3.2 kb DNA insert (vaIL) having an **alkaline lipase** gene according to the present invention;

FIG. 2 shows an agarose gel electrophoresis of the recombinant vector pHL1 having an **alkaline lipase** gene according to the present

invention, in which M denotes a size marker, lane 1 has a supercoiled type pUC19, lane 2 has a pUC19 digested with HindIII, lane 3 has a recombinant vector pHL1 digested with HindIII, the band of 2.7 kb corresponding to a vector pUC19 and the band of 3.2 kb corresponding to a DNA insert containing the **alkaline lipase** gene according to the present invention, and lane 4 has a supercoiled type recombinant vector pHL1; FIG. 3A shows an agarose gel electrophoresis of a DNA fragment containing the **alkaline lipase** gene according to the present invention, and FIG. 3B shows a photograph of Southern blotting, in which M denotes a size marker marked by DIG, lane 1 has *Vibrio metschnikovii* chromosomal DNA, lane 2 has *Vibrio metschnikovii* chromosomal DNA digested with HindIII, lane 3 has *Vibrio metschnikovii* chromosomal DNA digested with AvaI and EcoRI, lane 4 has pUC19 digested with HindIII, lane 5 has a supercoiled type recombinant vector pHL1, lane 6 has a recombinant vector pHL1 digested with HindIII, and lane 7 has recombinant vector pHL1/AvaI and EcoRI (probe); FIGS. 4A and 4B show a base sequence of a DNA insert containing the **alkaline lipase** gene from *Vibrio metschnikovii* RH530 N-4-8 according to the present invention, a regulatory element and an amino acid sequence derived therefrom; FIG. 5 shows a restriction enzyme map from which a minimum length and a gene position for expression of the **alkaline lipase** according to the present invention are identified in the DNA insert of the recombinant vector pHL1; FIG. 6 shows the comparison result of an amino acid sequence deduced from the **alkaline lipase** gene according to the present invention with *Pseudomonas glumae*, and *Burkholderia cepacia*; FIG. 7A shows a restriction enzyme map of a region prior to the promoter of the **alkaline lipase** gene according to the present invention, and FIG. 7B shows a change in activity when the region prior to the promoter is removed using the restriction enzyme; FIG. 8A shows a change in activity of the **alkaline lipase** according to the present invention, and FIG. 8B shows the measuring result of residual activity of the **alkaline lipase** according to the present invention depending on temperature; FIG. 9A shows a change in activity of the **alkaline lipase** according to the present invention depending on pH, and FIG. 9B shows the measuring result of residual activity of the **alkaline lipase** according to the present invention depending on pH; and FIG. 10 shows the effect of surfactant or detergent on the activity and stability of the **alkaline lipase** according to the present invention, for which enzyme solutions mixed with sodiumolefinsulfonate (AOS) (FIG. 10A), sodium alkylbenzen-sulfonate (LAS) (FIG. 10B) and sodium dodecyl sulfate (SDS) (FIG. 10C) are spotted on a 0.5% tricaprylin medium.

AB An **alkaline lipase** isolated from *Vibrio metschnikovii* RH530 and a polynucleotide sequence encoding the same are provided. The isolated **alkaline lipase** has an amino acid sequence of SEQ ID NO: 5 and the polynucleotide having a base sequence of SEQ ID NO: 4 encodes the **alkaline lipase**. The isolated **alkaline lipase** exhibits an optimal activity at a high pH level, that is, at pH 10-11, and has very high ratio of residual enzyme activity and high compatibility with a surfactant, so that it can be suitably used as an enzyme for a laundry detergent.

L2 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2004-05981 BIOTECHDS

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from *Vibrio metschnikovii* RH530 N-4-8;

recombinant enzyme production in *Escherichia coli*

AUTHOR: JIN G; JHON S; LEE H; RHO H

PATENT ASSIGNEE: CJ CORP

PATENT INFO: WO 2004001029 31 Dec 2003

APPLICATION INFO: WO 2003-KR1227 23 Jun 2003

PRIORITY INFO: KR 2002-35410 24 Jun 2002; KR 2002-35410 24 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-082499 [08]

AB DERWENT ABSTRACT:

NOVELTY - An **alkaline lipase** (I) isolated from **Vibrio metschnikovii** RH530 N-4-8 comprising a fully defined sequence of 185 amino acids (S1) as given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) polynucleotide (II) encoding (S1); (2) recombinant vector (III) comprising (II); (3) host cell (IV) transformed by (III); and (4) detergent comprising (I).

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) (claimed). Preferred Polynucleotide: (II) comprises a fully defined sequence of 555, 798 or 2578 base pairs as given in the specification. Preferred Recombinant Vector: (III) is pHL1, pHLB29 or pHA AH38.

USE - (I) is useful as an enzyme for laundry detergent (claimed).

ADVANTAGE - (I) has high residual enzyme activity and high compatibility.

EXAMPLE - Culture medium comprising tryptone, yeast extract, sodium chloride in sodium carbonate buffer was used for culturing **Vibrio metschnikovii** RH530 N-4-8, at 30 degreesC. The cells were collected and treated with lysozyme to lyse the cell. The resultant product was treated with phenol and chloroform to remove protein, and a precipitate was removed by centrifugation. A **Vibrio** chromosomal DNA was obtained from the supernatant. The obtained chromosomal DNA was cut with a restriction enzyme HindIII to be recombined with a cloning vector pUC19, followed by transforming *Escherichia coli* HB101, thus cloning a DNA fragment containing a 3.2 kb **alkaline lipase** gene. The resulting recombinant vector was referred to as a vector pHL1. After treatment with the restriction enzyme HindIII, an electrophoresis with 1% agarose gel was performed. The agarose gel electrophoresis showed that the **alkaline lipase** gene was cloned. To confirm that a DNA fragment containing an **alkaline lipase** gene derived from *V. metschnikovii*, which is contained in a recombinant vector pHL1, is identical with the gene from *V. metschnikovii*, Southern blotting was performed. DNA of 3.2 kb was treated with an exonuclease Bal31 to subclone the same in a minimum length required for expression of a lipase. Production of the lipase was confirmed by formation of a clear halo, and the result of subcloning showed that 2.6 kb DNA fragment was necessary for lipase activity. The recombinant vector containing such a gene having a minimum length was referred to as pHLB29. DNA of 2.6 kb fragment was subcloned in a direction opposite to that of a SmaI site of pUC19, and referred to as pHA AH38. Although the 2.6 kb DNA fragment was subcloned in a reverse direction relative to a lac promoter, pHA AH38 produced a clear halo at a tricaprillin culture medium, confirming that an **alkaline lipase** promoter existed in the 2.6 kb DNA fragment and the promoter used when it is transcribed from *E. coli*. (35 pages)

L2 ANSWER 3 OF 5 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ADH51272 protein DGENE

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** RH530 N-4-8.

INVENTOR: Jin G; Jhon S; Lee H; Rho H

PATENT ASSIGNEE: (CJCJ-N) CJ CORP.

PATENT INFO: WO 2004001029 A1 20031231

35p

APPLICATION INFO: WO 2003-KR1227 20030623

PRIORITY INFO: KR 2002-35410 20020624

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-082499 [08]

CROSS REFERENCES: N-PSDB: ADH51271

DESCRIPTION: **Vibrio metschnikovii** lipase chaperone protein.

AB The present sequence is the protein sequence of a putative chaperone protein, denoted Vall1, for an **alkaline lipase** ADH51273, denoted Vall2, of **Vibrio metschnikovii** RH530 N-4-8. It is encoded by an open reading frame that is controlled by the same promoter sequence as the lipase open reading frame. Homology comparisons suggested that Vall2 is a lipase while Vall1 is a lipase chaperone or is the product of an auxiliary gene for extracellular secretion. Lipase Vall2 can be obtained by recombinant production, especially in transformed *Escherichia coli* HB101 (pHL1) host cells. It exhibits maximum activity at 50-60 degrees C and pH 10-11, and has resistance against 0.07% sodium alkylbenzene-sulfonate, 0.1% sodium-alpha olefin sulfonate and 0.1% sodium dodecyl sulfate, showing it to be potentially useful as an additive for a laundry detergent.

L2 ANSWER 4 OF 5 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ADH51273 protein DGENE

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** RH530 N-4-8.

INVENTOR: Jin G; Jhon S; Lee H; Rho H

PATENT ASSIGNEE: (CJCJ-N)CJ CORP.

PATENT INFO: WO 2004001029 A1 20031231 35p

APPLICATION INFO: WO 2003-KR1227 20030623

PRIORITY INFO: KR 2002-35410 20020624

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-082499 [08]

CROSS REFERENCES: N-PSDB: ADH51271

DESCRIPTION: **Vibrio metschnikovii** alkaline lipase.

AB The present sequence is the protein sequence of an **alkaline lipase**, denoted Vall2, from **Vibrio metschnikovii** RH530 N-4-8. The lipase can be obtained by recombinant production, especially in transformed *Escherichia coli* HB101 (pHL1) host cells. It exhibits maximum activity at 50-60 degrees C and pH 10-11, and has resistance against 0.07% sodium alkylbenzene-sulfonate, 0.1% sodium-alpha olefin sulfonate and 0.1% sodium dodecyl sulfate, making it potentially useful as an additive for a laundry detergent.

L2 ANSWER 5 OF 5 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ADH51271 DNA DGENE

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** RH530 N-4-8.

INVENTOR: Jin G; Jhon S; Lee H; Rho H

PATENT ASSIGNEE: (CJCJ-N)CJ CORP.

PATENT INFO: WO 2004001029 A1 20031231 35p

APPLICATION INFO: WO 2003-KR1227 20030623

PRIORITY INFO: KR 2002-35410 20020624

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-082499 [08]

CROSS REFERENCES: P-PSDB: ADH51272; ADH51273

DESCRIPTION: **Vibrio metschnikovii** alkaline lipase gene.

AB The present sequence comprises a fragment of **Vibrio metschnikovii** RH530 N-4-8 chromosomal DNA found in vector pHL1. This vector was obtained by HindIII digestion of RH530 N-4-8 DNA and insertion of digested fragments into cloning vector pUC19. The sequence comprises 2 open reading frames, vall1 and vall2 (also claimed), existing under a single promoter. Homology comparisons suggested that vall2 encodes a lipase while vall1 encodes a lipase chaperone or is an

auxiliary gene for extracellular secretion. The lipase can be obtained by recombinant production, especially in transformed Escherichia coli HB101 (pHL1) host cells. It exhibits maximum activity at 50-60 degrees C and pH 10-11, and has resistance against 0.07% sodium alkylbenzene-sulfonate, 0.1% sodium-alpha olefin sulfonate and 0.1% sodium dodecyl sulfate, showing it to be potentially useful as an additive for a laundry detergent.

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